SECTION 11

GUIDELINES FOR FISH SAMPLING AND TISSUE PREPARATION FOR BIOACCUMULATIVE CONTAMINANTS

11.1 Introduction

11.1.1 Sampling of fish and shellfish for bioaccumulative contaminants has been conducted for over 35 years. Most fish sampling for contaminants has focused on contaminants of local concern, so data results and program conclusions have not always been comparable. The issues surrounding management of chemical contaminants in fish are of increasing concern for fishery management, environmental and public health agencies. The interdisciplinary multiagency problems caused by chemical contaminants suggests the need for standard sampling protocols. There have been inconsistent warnings given to the public by local, state, and federal regulatory agencies regarding the consumption of sport fish. This has been particularly evident on bodies of water shared by two or more states and on international waters. The Great Lakes States (Great Lakes Fish Consumption Advisory Task Force) and those States and EPA Regions bordering the Mississippi (Mid-America Fish Contaminants Group) and Ohio Rivers (Ohio River Valley Water Sanitation Commission) have endeavored to provide consistent sampling and advisory information but a standard protocol has yet to be agreed upon.

11.1.2 The application of quantitative risk assessment including hazard assessment, dose response assessment, exposure assessment and risk characterization functions best with a standardized protocol. The development of human health fish consumption advisories, whether based on quantitative risk assessment or some other methodology, is fundamentally affected by the procedures used in sampling. This section presents guidance for the sampling and preparation of fish for contaminant analysis, which is a key component of exposure assessment in quantitative risk assessment.

11.1.3 The purpose and goals of each study should be clearly stated prior to the initiation of fish collection for contaminant analysis. One should consider the overall long-term development of a fish contaminant database in each jurisdiction. Frequently short term goals have been the only consideration, where as long term trend assessments may provide a better understanding of the problem because the long.view is the only way of gauging important changes occurring in water quality.

11.1.4 Various federal, state, and local agencies have responsibilities for the collection and preparation of fish samples. Thus, numerous collection protocols are available. Fish sampling for contaminant analysis will often be included in other biological surveys to maximize use of the resource and to minimize costs. It must be recognized that any sample collected represents the future expenditure of significant dollar amounts by the time a decision is reached, and can have significant effects on major sectors of our society.

11.1.5 These guidelines present a basic fish sampling protocol designed to give comparable results between studies. Some additional requirements are pointed out which may be needed in special studies where different sizes or species of fish might be targeted or where special collections for spike samples might be needed. A partial discussion of sampling strategy including statistical concerns can be found in USEPA (1989), which should be reviewed during any planning effort.

11.2 Site Selection

11.2.1 Collecting sites should be established according to the specific requirements of each study. Sites may be designed as short- or long-term depending on the frequency with which they are sampled. Most sampling designs for short-term (synoptic) studies will be structured to determine the extent of contamination in a water body or a section of a water body. The determination of contamination gradients extending away from point sources or industrial/urban areas with point and non-point sources provides important information needed to manage contaminant burdens in fish. Some sites will be selected by individual states to address intrastate needs while other sites will be selected to address interstate needs through cooperative programs. Regardless of the various reasons for site selection, long-term comparability is of utmost importance to provide trend information needed to place bioaccumualtive contaminants in perspective.

11.2.2 Sites should be described as sport, commercial, or having both types of fisheries, and additional sites may be identified for ecological risk assessment. Special watershed information should be indicated, including urban areas, mining, manufacturing, agriculture, etc., and any known point or non-point sources of pollution at or near the site in the watershed. Additional information should include average width, depth, and velocity at the sampling station, description of the substrate, duration of the sampling effort, and habitat area sampled (e.g., length of stream or area of lake). Selected water quality measurements (e.g., conductivity, pH, dissolved oxygen, temperature, etc.) may also be useful. It is becoming routine to collect and analyze water, sediment and fish at common stations to gain a more complete understanding of contaminants in aquatic environments.

11.3 Sample Collection

11.3.1 The following three objectives should guide sample collection:

1. Provide comparable data

2. Utilize sizes and ages of species generally available to the fishery and,

3. Yield data which will screen for problems that might indicate that more intensive studies are needed.

11.3.2 Samples should be obtained at each station from the principal fish categories. Fish species are grouped by feeding strategy into predators, omnivores and bottom feeders. To reduce the number of categories, the

omnivores may be placed with the bottom feeders. USEPA (1990a) sampled 388 sites nationwide at which 119 different species of fish representing 33 taxonomic families of fish were collected. The most frequently sampled freshwater and marine species in that study are listed in Table 1.

11.3.3 This national study indicates that of the freshwater species, carp and largemouth bass were the most frequently sampled and are the most likely to provide interstate comparability. The other freshwater species listed may be selected in a declining order of priority; however, additional less common species may not be added except in special situations. The diversity of marine species is much greater resulting in a lack of focus on a limited number. Additional effort will be needed to determine which marine species should receive priority on the Atlantic, Pacific and Gulf Coasts in order to provide long term comparative data.

11.3.4 Cunningham et al. (1990) in a census of state fish/shellfish consumption advisory programs found that approximately 60 species of fish and shellfish are used as the basis for consumption advisories nationwide. The leading fish families are the Ictaluridae (catfish), Centrarchidae (sunfish, largemouth and smallmouth bass), Cyprinidae (carp), and Salmonidae (salmon and trout). Among shellfish, crustaceans (e.g., blue crab) and molluscs (e.g., American oyster, soft-shelled clam, and blue mussel) are the most widely used. The criteria most frequently used for collecting fish/shellfish species were: 1) the dominant species harvested for consumption, 2) the most abundant species and 3) the species representing a specific trophic order.

11.3.5 Consistent sampling of common species over long time periods (several years) and large geographic areas will greatly facilitate future trend analyses. Many species are similar in appearance, and taxonomic identification must be reliable to prevent mixing species. Under no circumstance should two or more species be mixed to create a composite sample. Fish for contaminant analyses may be obtained during studies to determine fish community structure. The measurement of multiple parameters (e.g., fish health condition assessment, histopathological examination, bioindicators of stress, etc.) are encouraged on common samples to provide the information needed in ecological risk assessment.

11.3.6 Screening studies should endeavor to collect the largest individuals available. However, more detailed studies should sample the predominant two or three age classes of the same species in a water body to determine the relationship between contaminant burden and fish size (age) to provide information needed for greater risk management flexibility. This information could allow the lifting of an advisory on smaller, more abundant sizes of a contaminated species with lower body burdens if these were important to a sport fishery.

11.3.7 The frequency of sampling should be considered in each study design. Most long-term monitoring programs will be based on an annual frequency due to the costs of analysis. However, special studies may require seasonal sampling. Fish sampled in the fall may tend to have a higher lipid content than those sampled during the spring. Sampling freshwater in the spring may

TABLE 1. FREQUENCY OF OCCURRENCE FOR FRESHWATER AND MARINE SPECIES IN THE NATIONAL FISH BIOACCUMULATION STUDY (USEPA, 1990a)

FRESHWATER

Bottom Feeder Species	Site Occurrence
Carp	135
White sucker	32
Channel Catfish	30
Redhorse sucker	16
Spotted sucker	10

Game (Predator) Species	Site Occurrence
Largemouth Bass	83
Smallmouth Bass	26
Walleye	22
Brown trout	10
White Bass	10
Northern Pike	8
Flathead Catfish	8
White Crappie	7
Rainbow trout	7

MARINE

Species	Site Occurence
Hardhead catfish	7
Starry flounder	5
Blue fish	5
White perch	4
Winter flounder	4
White sturgeon	4
Red drum	3
Black drum	3
Striped mullet	3
Atlantic croaker	3
Spot	3
Spotted seatrout	3
Weakfish	3
Sheepshead	2
Southern flounder	2
Flathead sole	2
Atlantic salmon	2
Red snapper	2
Gizzard shad	1
Atlantic cod	1
Yellow jack	1
Striped bass	1
American shad	1
Surf smelt	1
Spotted drum	1
Crevalle jack	1
Redstripe rockfish	1
Summer flounder	1
Diamond turbot	1
Hornyhead turbot	1
Bocaccio	1
White surfperch	1
Quillback rockfish	1
Brown rockfish	1
Copper rockfish	1
American eel	1

find fish more available due to spawning movements exhibited by spring spawning species; however, extensive movement may temporarily dislocate fish from the usual area where they have been exposed to contaminants. The various methods of collecting fillets (skin-on versus skin-off, belly flap included or excluded) must be standardized. A skin-on fillet with belly flap included is recommended. A lipid analysis of each sample is required for trend analysis and model validation, however, lipid content is not recommended for use in normalizing the differences among fillet types because it frequently increases the variance in the data (NOAA, 1989). Even when considering the bioaccumulation of lipophilic compounds all of the compound is typically not stored in the lipid. At any given time additional amounts of the compound will be found in the cell moisture and the non-lipid tissue. Lipid content may also provide insight into seasonal changes within species, as well as identify differences between species used in contaminants monitoring.

11.3.8 Active sampling techniques (electrofishing, trawling, seining, etc.) are preferred over passive capture techniques (gill nets, trammel nets, etc.) however, the latter can be used as long as the gear is checked on a frequent basis to avoid sample deterioration. Species that are difficult to collect may be obtained from a commercial fisherman, but only when the collector accompanies the fisherman to verify the time and place of capture. Following collection, fish should be placed on wet ice in clean coolers prior to processing. Fish should be either processed within 24 hours or frozen within 24 hours for later processing if immediate processing is not possible. If analyses of fish eggs or internal organs are required, a sample size of at least 20 grams is required.

11.3.9 Composite samples of three to ten fish (same species) are recommended for each of the predator and bottom feeder categories based on the variability of contaminant concentration in fish at the site. The number of fish/composite selected should remain constant over time and space for each species monitored. Composites are used to reduce the cost of analysis per fish; however, it must be recognized that statistical manipulation of the data is compromised when individual values are not determined. The smallest size fish in a composite should equal 75% of the total length of the largest fish in a composite, e.g., if the largest is 400 mm, the smallest should not be less than 300 mm. Replicate composite samples may be added as needed to meet statistical requirements; (USEPA, 1989) however, the cost of additional samples will quickly become a factor. The most important sport and/or commercial species in each feeding strategy group should be used for analysis. Composite samples can be collected for either fillet analysis (human health risk assessments) or for whole body analysis (ecological risk assessments and worst case monitoring).

11.3.10 When a study is planned, it is not certain that the quantity of each species indicated for analysis can be obtained especially if the water body has had little or no prior sampling activity. In order to meet both the human health and ecological requirements a sample of a sport fish species and a bottom feeder species is needed. The sport fish species is usually filleted and the data used for human health risk assessment. The whole body analysis of bottom feeder species is used both for initial "worst case" monitoring and for ecological risk assessment.

11.3.11 If fish are not abundant or detailed comparisons with other parameters are desired, it may be possible to do a reconstructed analysis (Figure 1) on a single species either sport fish or bottom feeder. To do a reconstructed analysis, the fish are filleted and the remainder of the carcass is saved for analysis. The contaminant concentrations in both the fillet and remaining carcass portions can then be added together to estimate the whole body concentration. A lipid analysis must be performed on both the fillet and remaining carcass to allow normalization of the contaminant concentrations in both samples. A reconstructed analysis may be performed on either single fish or composite fish samples, however, the data may be more reliable if single fish are analyzed.

11.3.12 Sediment samples can sometimes indicate a "hot spot" and can be helpful in determining the source(s) of contamination or the zones of deposition. However, sediment samples cannot be used as a substitute for fish collections, but both can provide complimentary data.

11.4. Sample Preparation For Organic Contaminants in Tissue

11.4.1 Collection Precautions

11.4.1.1 In the field, sources of tissue contamination include sampling gear, boats and motors, grease from ship winches or cables, engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. For example, to avoid contamination from ice, the whole samples (e.g., molluscs in shell, whole fish) should be wrapped in aluminum foil, placed in watertight plastic bags, and immediately cooled in a covered ice chest. Many sources of contamination can be avoided by resecting (i.e., surgically removing) tissue in a controlled environment (e.g., a laboratory). Organisms should not be frozen prior to resection if analyses will be conducted on only selected tissues (e.g., internal organs) because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue may be wrapped as described above and frozen. Tissue sample collection and preparation requirements are summarized in Table 2 (Puget Sound Estuary Program, 1989).

11.4.2 Processing

11.4.2.1 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed in isopropanol, and finally rinsed with organic free distilled water. Work surfaces should be cleaned with isopropanol, washed with distilled water and allowed to dry completely.

11.4.2.2 The removal of biological tissues should be carried out by or under the supervision of an experienced biologist. Tissue should be removed with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene (plastic) surfaces and implements are a

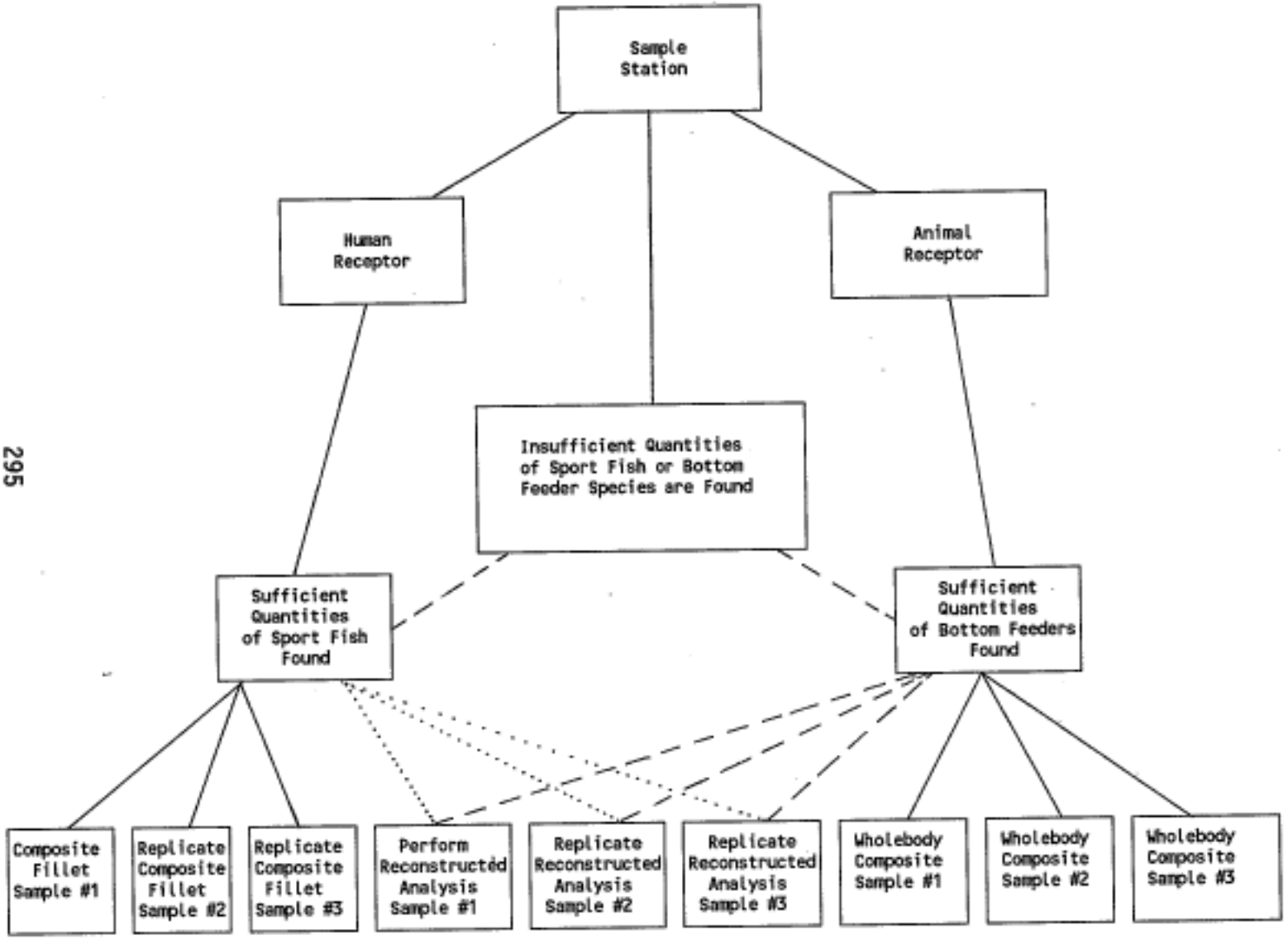

Figure 1. General sampling scheme for bioaccumulative contaminants in fish, multiple age groups will require additional samples.

TABLE 2. SUMMARY OF SAMPLE COLLECTION AND PREPARATION QA/QC REQUIREMENTS FOR FISH TISSUE (MODIFIED FROM PUGET SOUND ESTUARY PROGRAM, 1986, 1989)

Variable	Sample Size (a)	Container (b)	Preservation	Maximum Holding Time (c)	Maximum Extract Holding Time
Organic Compounds					
Wholebody Tissues (after resection)	--	A	Freeze (-18°C)	1 yr	40 days
Semivolatiles	25 g	G,T,A	Freeze (d) (-18°C)	1 yr	40 days
Volatiles	5 g	G,T	Freeze (d) (-18°C)	14 days	--
Trace Metals					
Wholebody Tissues (after resection)	--	W,P,B	Freeze	6 mo	
All Metals	5 g	P,B	Freeze (d)	6 mo	
(except Hg)	0.2 g	P,B	Freeze (d)	28 days	

a. Recommended wet weight sample sizes for one laboratory analysis. If additional laboratory analyses are required (i.e., replicates) the field sample size should be adjusted accordingly. If specific organs are to be analyzed, more tissue may be required.

b. G = glass, A = wrapped in aluminum foil, placed in watertight plastic bags, T = PTFE (Teflon), P = linear polyethylene, B = borosilicate glass, W = watertight plastic bags.

c. This is a suggested holding time. No USEPA criteria exist for the preservation of this variable.

d. Post-dissection

potential source of contamination and should not be used. To control contamination when resecting tissue, technicians should use separate sets of utensils for removing outer tissue and for resecting tissue for analysis.

11.4.3 Preparation of Composite Fillet Samples

11.4.3.1 For fish samples, special care must be taken to avoid contaminating targeted tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The proper handling in the preparation of fish tissue samples to decrease the likelihood of contamination cannot be over emphasized. To reduce variation in sample preparation and handling, samples should be prepared in the laboratory rather than in the field. However, if no laboratory is available, field preparation is acceptable if portable tables are used, dust and exhausts are avoided and proper decontamination procedures are followed. Regardless of where preparation occurs, the following subsections should be followed to insure quality fillet samples:

11.4.3.2 To initiate processing, each fish is measured (total or fork length) to the nearest tenth of a centimeter, weighed (nearest gram) and external condition noted. A few scales should be removed from each fish for age and growth analysis. This presents an excellent opportunity to systematically evaluate each fish using the Fish Health and Condition Assessment Methods (Section 10). Fish are scaled (or skinned: catfish) and filleted carefully, removing bones, to get all of the edible portion flesh.

11.4.3.3 A fillet includes the flesh tissue and skin from head to tail beginning at the mid-dorsal line from the left side of each fish and including the belly flap. The fillet should not be trimmed to remove fatty tissue along the lateral line or belly flap. A comparable fillet can be obtained from the right side of the fish and can be composited with the left fillet, kept separate for duplicate quality assurance analysis, analyzed for different compounds or archived. Each right and left fillet should be weighed individually, recorded and individually wrapped in clean aluminum foil.

11.4.3.4 Care must be exercised not to puncture any of the internal organs. If the body cavity is entered, rinse the fillet with distilled water. Fish sex and condition of internal organs are determined during or after filleting. This skin-on fillet deviates from the skin-off fillets analyzed in the National Fish Bioaccumulation Study (USEPA 1990a), however, skin-on is recommended because it is believed that this is the way most sport anglers prepare their fillets. The issue of skin-on versus skin-off fillets differs greatly among jurisdictions (Hesse, 1990) and is far from settled, however, the above recommendations appear to be the preferred method unless the species specificity is increased in future guidelines.

11.4.3.5 Filleting should be conducted on cutting boards covered with heavy duty aluminum foil, which is changed between composite samples. Knives, fish scalers, measurement boards, scales, etc. should be cleaned with reagent grade isopropanol, followed by a rinse with distilled water between each composite sample.

11.4.3.6 Because of the low limits of detection for many environmental analyses, clean field and laboratory procedures are especially important. Sample contamination can occur during any stage of collection, handling, storage or analyses. Potential contaminant sources must be known and steps taken to minimize or eliminate them.

11.4.3.7 Large sheets of heavy duty aluminum foil should be used to carefully fold and completely wrap the fillet samples. When filling out I.D. labels use pencil or waterproof marker and place the foil wrapped sample in a secured plastic bag.

11.4.4 Storage

11.4.4.1 Recommended holding times for frozen tissue samples have not been established by USEPA, but a maximum 1 year holding time is suggested. For extended sample storage, precautions should be taken to prevent desiccation. National Institute For Standards and Technology is testing the effects of long-term storage of tissues at temperatures of liquid nitrogen(-120° to -190°C). At a minimum, the samples should be kept frozen at -20°C until extraction. This will slow biological decomposition of the sample and decrease loss of moisture. Liquid associated with the sample when thawed must be maintained as part of the sample because the lipid tends to separate from the tissue. Storage of samples should remain under the control of the sample collector until relinquished to the analytical laboratory.

11.4.4.2 Whole fish may be frozen and stored if no resection of internal organs or fillets will be conducted and the ultimate analysis is whole body. However, if resection of fillets or organs is required, these tissues should be removed prior to freezing and can be stored frozen in appropriate individual containers. The tissues may then be ground and homogenized at a later date and refrozen in sample packets for shipment on dry ice to the analytical laboratory(s).

11.4.4.3 It is frequently necessary to ship whole fish, fillets or homogenized tissue samples over long distances to an analytical laboratory. To avoid sample deterioration, it is recommended that all samples be frozen solid prior to shipment. The frozen and logged samples should be wrapped in newspaper to provide additional insulation for the samples which are shipped in well sealed insulated containers with an appropriate quantity of dry ice. The quantity of dry ice should be sufficient to eliminate any defrosting of the samples during the time of priority transport. However, in the event that a delay occurs in transit, these recommendations will provide some assurance that the samples will arrive in usable condition. Under no circumstances should unfrozen tissue be shipped either with or without dry ice because the quality of the sample cannot be assured.

11.4.5 Tissue Preparation

11.4.5.1 Organic contaminants are not evenly distributed throughout biological tissue, especially in fish. This is also true for fish fillets. Therefore, to obtain a homogenous sample, the whole fish or the whole fillet

must be ground to a homogeneous consistency. This procedure should be carried out by the sample collector on partially thawed samples.

11.4.5.2 Chop the sample into 2.5 cm cubes unless the sample is small enough to fit in a hand crank meat grinder (300 gm or less) or a food processor (Hobart Model 8181D or equivalent for large fish) (USEPA, 1990b). Then pass the whole sample through a meat grinder. Grinding of biological tissue is easier when the tissue is partially frozen. This is especially true when attempting to grind the skin. Chilling the grinder with a few chips of dry ice will reduce the tendency of the tissue to stick to the grinder. Do not freeze the grinder since hard frozen tissue is difficult to force through the chopper plate.

11.4.5.3 The ground sample is divided into quarters, opposite quarters are mixed by hand with a clean stainless steel spatula and then the two halves are mixed back together. Repeat the mechanical grinding, quartering and hand mixing two more times. No chunks of tissue should be present at this point as they will not be efficiently extracted. Very small fish or small fillets may be homogenized in a high speed blender.

11.4.5.4 When compositing fillets or whole fish each individual fillet or fish should be ground separately following the above described procedure. Then take equal amounts from each fillet or fish sample to be composited to provide a total equal to that required for extraction or the total number of split and archived samples required by the study plan.

11.4.5.5 If the ground fish is to be re-frozen prior to extraction and analysis, weigh out the exact amount for extraction into a small container. Using a top loading balance, tare a 2 oz. glass jar (or a small sheet of aluminum foil that can be formed into a sealed packet) to 0.0 gm and carefully dispense a 20.0 gm portion of homogenized tissue into the container. Tightly seal the container or foil packet. Repeat with additional containers for duplicates, splits, or archived samples. Lipid material tends to migrate during freezing; therefore, storing a weighed portion ensures extraction of a representative portion of the tissue if the foil or container is completely rinsed with solvent by the analytical chemist.

11.4.5.6 Whenever a ground sample is to be split between two or more labs, the ground sample must also be mixed with reagent grade anhydrous sodium sulfate (previously heated to 400°C to drive off any phthalate esters acquired during storage). To ensure the homogeneity of the sample prior to splitting, transfer 100 gm of ground tissue to a 600 mL beaker. Add 250 gm of anhydrous sodium sulfate and mix thoroughly with a stainless steel spoon or a spatula. There should not be any lumps and the mixture should appear homogeneous. Dispense exactly 70.0 gm of mixture to each lab and note on the package that it contains 20 gm of tissue.

11.4.5.7 When preparing the tissue for volatile analysis, grind it in an area free of volatile organic compounds. The meat grinder or food processor must be heated in an oven for 30 minutes at 105°C after solvent rinsing and then allowed to cool at room temperature. Immediately after grinding the tissue,

weigh duplicate 1 gm portions into culture tubes with screw caps. Analyze immediately or store in a freezer.

11.5 Sample Preparation For Metal Contaminants In Tissue

11.5.1 Collection Precautions

11.5.1.1 The major difficulty in trace metal analyses of tissue samples is controlling contamination of the sample after collection. In the field, sources of contamination include sampling gear, grease from winches or cables, engine exhaust, dust, or ice used for cooling. Care must be taken during handling to avoid these and any other possible sources of contamination. For example, during sampling the ship should be positioned such that the engine exhausts do not fall on deck. To avoid contamination from melting ice, the samples should be placed in watertight plastic bags.

11.5.1.2 Sample resection and any subsampling of the organisms should be carried out in a controlled environment (e.g., dust-free room). In most cases, this requires that the organisms be transported on ice to a laboratory rather than being resected in the field. It is recommended that whole organisms not be frozen prior to resection if analyses will be conducted only on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue (e.g., muscle) may be wrapped as described above and frozen (Puget Sound Estuary Program, 1986).

11.5.1.3 Resection is best performed under "clean room" conditions. The "clean room" should have positive pressure and filtered air and also be entirely metal-free and isolated from all samples high in contaminants (e.g., hazardous waste). At a minimum, care should be taken to avoid contamination from dust, instruments, and all materials that may contact the samples. The best equipment to use for trace metal analyses is made of quartz, TFE (tetrafluoroethylene), polypropylene, or polyethylene. Stainless steel that is resistant to corrosion may be used if necessary. Corrosion-resistant stainless steel is not magnetic, and thus can be distinguished from other stainless steels with a magnet. Stainless steel scalpels have been found not to contaminate mussel samples (Stephenson et al., 1979). However, low concentrations of heavy metals in other biological tissues (e.g., fish muscle) may be contaminated significantly by any exposure to stainless steel. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass would be preferred over plastic if trace organic analyses are to be performed on the same sample.

11.5.1.4 Resection should be conducted by or under the supervision of a competent biologist. Special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The procedure previously outlined for the preparation of fillet samples should generally be followed. Unless specifically sought as a sample, the dark muscle tissue that may exist in the

vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass.

11.5.1.5 Prior to use, utensils and bottles should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. For quartz, TFE, or glass containers, use 1+1 HNO_3, 1+1 HCl, or aqua regia (3 parts conc. HCl + 1 part conc HNO_3) for soaking. For plastic material, use 1+1 HNO_3 or 1+1 HCl. Reliable soaking conditions are 24 h at 70°C (APHA, 1989; 1992). Do not use chromic acid for cleaning any materials. Acids used should be at least reagent grade. For metal parts, clean as stated for glass or plastic, except omit the acid soak step. If trace organic analyses are to be performed on the same samples, final rinsing with methylene chloride is acceptable.

11.5.1.6 Sample size requirements can vary with tissue type (e.g., liver or muscle) and detection limit requirements. In general, a minimum sample size of 6 g (wet weight) is required for the analysis of all priority pollutant metals. To allow for duplicates, spikes, and required reanalysis, a sample size of 50 g (wet weight) is recommended. Samples can be stored in glass, TFE, or high-strength polyethylene jars.

11.5.2 Processing

11.5.2.1 Samples should be frozen after resection and kept at -20°C. Although specific holding times have not been recommended by USEPA, a maximum holding time of 6 months (except for mercury samples, which should be held a maximum of 28 days) would be consistent with that for water samples.

11.5.2.2 When a sample is thawed, the associated liquid should be maintained as a part of the sample. This liquid will contain lipid material. To avoid loss of moisture from the sample, partially thawed samples should be homogenized. Homogenizers used to grind the tissue should have tantalum or titanium parts rather than stainless steel parts. Stainless steel blades used during homogenization have been found to be a source of nickel and chromium contamination. Some trace metal contamination during processing cannot be avoided and it is therefore necessary to determine and control the amount of contamination introduced during processing. Contamination can be monitored by introducing a dry ice blank into the blender and analyzing the chips.

11.5.2.3 To avoid trace metal contamination during processing the preferred method is to proceed to a chemical digestion process which minimizes or eliminates resection, homogenization, or grinding. Chemical digestion is best limited to specific organ tissues from large fish or to smaller sized whole fish.

11.6 Identification of Composite Whole Fish or Fillet Samples

11.6.1 Composite whole fish samples will be made up of three to ten fish with any deviation in number clearly identified. The limitation on the variance between individual fish in each composite will be as previously described. The length and weight of each fish must be recorded. The same field

information should be provided as described above for both fillet and/or whole body composite samples. The same handling precautions as described above should be followed for either organic or trace metal contaminants. Spines on whole fish should be sheared to minimize puncturing the sample packaging.

11.6.2 The following information should be included on the field/lab form for each sample collected:

11.6.2.1 Project Name

11.6.2.2 Station Code (if applicable)

11.6.2.3 Date

11.6.2.4 Collector's Name

11.6.2.5 Sampling location (river mile and/or other specific information relating to local landmarks)

11.6.2.6 Latitude and Longitude

11.6.2.7 Water body name

11.6.2.8 Sampling technique(s), i.e. 230 vac electrofishing apparatus, hoop nets, etc.

11.6.2.9 Fish species

11.6.2.10 Individual lengths and weights of fish in sample

11.6.2.11 Sample type (Whole or Fillet)

11.6.2.12 Individual fillet weights (whether left or right)

11.6.2.13 Comments or Unusual Conditions, i.e., tumors, sores, fin rot, blind, etc.

11.7 Chain-of-Custody Procedures (USEPA, 1990c; USEPA, 1991) Also See Section 2, Quality Assurance and Quality Control.

11.7.1 All samples should be kept in a secure (locked) area to avoid legal complications in administrative proceedings. Transportation of the samples must be coordinated between the agency responsible for the field collection and the agency responsible for analytical work. When custody of the samples is transferred, the following checks should be implemented:

11.7.1.1 All transfers should be properly relinquished to ensure chain-of-custody. Transfers should be recorded on a form separate from the field data sheet. The chain-of-custody form should include the sample identification number(s). Custody tags must be used and numbered in sequence (if possible).

11.7.1.2 The field data sheet should stay with the sample until it is logged in by the analytical laboratory.

11.7.1.3 Samples can be shipped and chain-of-custody maintained as long as shipping containers are sealed with custody tape.

11.7.1.4 Samples should remain frozen until they are prepared for analysis. Shipping with dry ice is recommended.

11.7.1.5 The laboratory's receiving agent should initial the field data sheet and affix the date of sample receipt. Depending on administrative need, a copy of this form (with initials and date of sample receipt plainly visible) may be required by the lab agency's central office.

11.8 Conclusion

11.8.1 This protocol only addresses the steps to be considered in field sampling fish and sample preparation for human health fish consumption advisories and ecological risk assessment. Additional protocols must be followed to carry out the appropriate analytical chemistry and the risk assessment/management requirements leading to an action. These additional protocols were beyond the scope of this assignment.

11.9 Literature Cited

Cunningham, P.A., J.M. McCarthy and D. Zeitlin 1990. Results of the 1989 Census of State Fish/Shellfish Consumption Advisory Programs. Prepared for S.M. Kroner, Assessment and Watershed Protection Division, OWRS, USEPA, by Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC.

APHA. 1989. Standard methods for examination of Waste and Wastewater. 17TH Ed. American Public Health Association, Washington, DC.

APHA. 1992. Standard methods for examination of Waste and Wastewater. 18TH Ed. American Public Health Association, Washington, DC.

Hesse, John L. Michigan Department of Public Health, 1990. Summary and Analysis of Existing Sportfish Consumption Advisory Programs in the Great Lakes Basin. The Great Lakes Fish Consumption Advisory Task Force Co-Chaired by H.A. Anderson and L. Liebenstein, State of Wisconsin. Unpublished.

NOAA. 1989. A summary of data on tissue contamination from the first three years (1986-89) of the mussel watch project. Technical Memorandum, NOS, OMA49. Rockville, MD.

Puget Sound Estuary Program 1986. Recommended Protocols for Measuring Metals in Puget Sound Water, Sediment and Tissue Samples. Prepared by Tetra Tech, Inc., Bellevue, WA. *In*: Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound. USEPA, Region 10, Seattle, WA (Looseleaf).

Puget Sound Estuary Program 1989 (Revised). Recommended Guidelines for Measuring Organic Compounds in Puget Sound Sediment and Tissue Samples. Prepared by Tetra Tech, Inc., Bellevue, WA. *In*: Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound. USEPA, Region 10, Seattle, WA (Looseleaf).

Stephenson, M.D., M. Martin, S.E. Lange, A.R. Flegal and J.H. Martin 1979. California Mussel Watch 1977-78. Volume II: Trace metals concentrations in the California mussel, *Mytilus californianus*. SWRCB Water Quality Monitoring Report No. 79-22. Sacramento, CA.

USEPA, 1989. Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish: A Guidance Manual. EPA-503/8-89-002. Office of Marine and Estuarine Protection and Office of Water Regulations and Standards, Washington, DC.

USEPA, 1990a. Bioaccumulation of Selected Pollutants in Fish, A National Study Volume I and II. EPA-506/6-90/001. Office of Water Regulations and Standards (WH-552), Washington, DC.

USEPA, 1990b. Extraction and Analysis of Organics in Biological Tissue, Method OB 8/90, USEPA, Environmental Services Division, Region IV, Analytical Support Branch, Athens, GA.

USEPA, 1990c. Manual for the certification of laboratories analyzing drinking water. Criteria and procedures quality assurance. EPA/570/9-90/008. Prepared by the Laboratory Certification Program Revision Committee. Office of Water (WH-550D), Washington, DC.

USEPA, 1991. Manual for the evaluation of laboratories performing aquatic toxicity tests. EPA/600/4-90/031. Klemm, D.J., L.B. Lobring, and W.H. Horning, II. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.